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Hydrophilic gel containing coenzyme Q_{10}-loaded liposomes: preparation, characterization and stress stability tests

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The aim of this study was to develop a semisolid formulation containing liposomes loaded with coenzyme Q_{10} (Q_{10}). Q_{10}-loaded liposome dispersion prepared from non-hydrogenated lecithin and characterized for particle size, polydispersity index (PDI), pH value and Q_{10}-content was incorporated into carbomer gel, and a liposome gel was obtained. Liposome gel and liposome-free gel were analyzed for flow properties by continuous rheology measurements, pH values and Q_{10}-content, 48 h after preparation and after a temperature stress test (1 cycle: 24 h at 4ºC, 24 h at 20±2ºC and 24 h at 40ºC), in order to predict their long-term stability. Liposomes were identified in liposome dispersion and liposome gel by freeze fracture electron microscopy (FFEM), while their particle size, PDI and zeta potential were determined by photon correlation spectroscopy (PCS). Q_{10}-loaded liposomes were of small particle size (125 nm), homogeneous (PDI=0.2) and negatively charged, and their incorporation into the gel did not significantly change ($p>0.05$) their particle size and PDI. FFEM confirmed liposomes presence in the liposome gel. Liposome and liposome-free gel revealed non-Newtonian, shear-thinning plastic flow behavior. The temperature stress test revealed that temperature changes did not significantly influence ($p>0.05$) the pH value, while they significantly decreased ($p<0.05$) Q_{10}-content in gels. Q_{10} was significantly more stable ($p<0.05$) in liposome gel than in liposome-free gel. Rheological parameters of liposome-free gel significantly changed, in contrast to the liposome gel. In conclusion, Q_{10}-loaded liposome gel suitable for dermal use was developed, exhibiting high stability even after subjecting to the temperature stress test.

Keywords*:* Liposome, Coenzyme Q_{10}, Carbomer gel, Stability, Rheology

INTRODUCTION

The application of coenzyme Q_{10} (Q_{10}) in pharmaceutical industry has grown significantly in the past decade, i.e., it has been successfully applied in medicine, cosmetics and nutraceuticals [1]. Coenzyme Q_{10} is a highly effective liposoluble non-enzymatic antioxidant which represents the first line of antioxidant defense [2]. Q_{10} has been known since 1950, but became commercially available since its isolation from tobacco plants. Biotechnological or synthetic production is nowadays possible [2].

Q_{10} has radical scavenger and bioenergetical properties. It is already known for its efficacy in the area of neurodermatitis, psoriasis, periodontitis, external substitution under stress, adiposity, immune support and has many other benefits [3]. Q_{10} is highly effective in protecting keratinocytes from DNA damage induced by UVA radiation and also in preventing photoageing *in vivo* with a reduction in wrinkle depth [4]. Because of its beneficial effects, i.e. its antioxidant activities against environmental aggressions and photoageing, Q_{10} has recently made its way into many pharmaceutical and cosmetic products. However, Q_{10} can be easily oxidized, especially under aerobic conditions and light exposure. In order to enhance the photostability of Q_{10}, it may be incorporated into different nanocarriers. Incorporation of Q_{10} into nanostructured lipid carriers (NLC) significantly improved the photo-stability of Q_{10} [5]. In addition, since Q_{10} is liposoluble and hence complicated to incorporate into cosmetic formulations, its incorporation into nanocarriers, like liposomes, nanoemulsions, nanoparticles, would facilitate its use in formulating cosmetic products. Generally, Q_{10} can be incorporated into various novel drug delivery carriers, which include liposomes, polymeric nanoparticles, polymeric micelles, solid lipid nanoparticles, nanostructured lipid carriers, self-emulsifying drug delivery systems, nanoemulsions, solid and aqueous dispersions [1]. Moreover, the incorporation of Q_{10} into nanocarriers would enhance its penetration into the skin, as it has been

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shown for Q_{10} encapsulated in solid lipid nanoparticles (SLN) being further incorporated in a carbomer gel [6]. The skin delivery of Q_{10} was doubled with gels containing Q_{10}-loaded SLN in comparison with gels prepared with only Q_{10}. For cosmetic purposes most cosmetic actives should be delivered into the skin in order to exhibit their effect. Hence, the use of nanocarriers loaded with cosmetic actives has proven to be adequate as they deliver the actives into the skin, i.e. even into the dermis. Nanostructured lipid carriers (NLC) with a size of about 230 nm have shown to be beneficial for the dermal delivery of Q_{10} and they increased Q_{10} skin penetration when compared to an equally sized nanoemulsion [7], which was in accordance with results reported by Chen *et al.* [8], who showed an epidermal uptake of Q_{10} from Q_{10}-NLC being 10-11 times higher compared to that achieved by the Q_{10}-emulsion, proving that NLC are a promising carrier for the topical delivery of Q_{10}. Furthermore, dependent on the drug delivery system, also transdermal delivery of Q_{10} was reported. Namely, a tocopheryl phosphate mixture which self-assembles to form vesicular structures in hydroethanolic solutions (mean size between 101-162 nm) increased the permeation of carnosine, vitamin D3, caffeine and coenzyme Q_{10} into or through the skin [9]. As to the penetration enhancing ability, liposomes have been widely used to enhance dermal and transdermal drug delivery [10-17]. In brief, liposomes are small, spherical vesicles consisting of amphiphilic lipids, enclosing an aqueous core that are still highly appreciated due to some advantages over other encapsulation technologies [18]. A variety of drugs can be entrapped within liposomes. Topical delivery of liposomally encapsulated actives may offer advantages over conventional formulations since liposomes have the potential to: (a) reduce serious side-effects and incompatibilities that may arise from undesirably high systemic absorption of drugs, (b) act as a local depot for sustained release of dermally active components, (c) serve as penetration enhancers and (d) serve as a rate-limiting membrane barrier for the modulation of systemic absorption of drugs [19]. Several independent studies have shown a higher stability against UV radiation of vitamins encapsulated in liposomes compared to conventional formulations [20-22]. An improved stability was also found in the case of retinol and ascorbyl palmitate encapsulated in liposomes stored at room temperature compared to traditional formulations [23-24].

The purpose of this study was the development, characterization and stability evaluation of a semisolid formulation containing Q_{10}-loaded liposomes. Liposome dispersions are not used in their pure form in cosmetic formulations because of their liquid nature and are therefore incorporated in semisolids. In this study, as it presents our first study on semisolids containing Q_{10}-loaded liposomes, we used a hydrophilic gel as a base (vehicle) for the incorporation of liposomes. The gel (Ultrez® 10 polymer gel) was chosen as liposomes exhibit highest stability in gels, i.e. in creams containing emulsifiers they could be degraded. Thus, at first obtained liposomes, after their preparation (24 h after preparation) were characterized for their particle size, polydispersity index (PDI), pH value and Q_{10}-content. The obtained liposome gel and the liposome-free gel were analyzed for flow properties, pH value and Q_{10}-content, 48 h after their preparation and after five cycles of temperature stress. The liposome gel was also analyzed for the presence of liposomes and their particle size and homogeneity.

MATERIALS AND METHODS

Materials

The following substances were used: non-hydrogenated soybean lecithin (Phospholipon® 80, Lipoid GmbH, Germany), ubichinon (coenzyme Q_{10}, Gfn-Selco, Germany), carbomer (Ultrez 10 ® Polymer, Lubrizol, USA), phenoxyethanol (and) methylparaben (and) ethylparaben (and) propylparaben (and) butylparaben (Phenonip™, Clariant, Switzerland), diazolidinyl urea (Germall™II, Ashland, USA), propylene glycol (BASF, Germany), potassium dihydrogenphosphate (Sigma-Aldrich, USA), sodium hydroxide (Sigma-Aldrich, USA), triethanolamine (TEA) (Sigma, USA), edetate disodium (Titriplex III, Merck Millipore, USA). The water used was double distilled.

Preparation and characterization of Q_{10}-loaded liposomes

Preparation of liposomes. Liposomes composed of Phospholipon® 80 (PL 80) were prepared by the following method: Q_{10} (0.5 % w/w) was dissolved in a mixture of ethanol (16 % w/w) and Phospholipon® 80 (10 % w/w) at 50°C and the obtained solution was added at room temperature to a phosphate buffer solution (PBS) pH 6.5 under vigorous stirring (10 000 rpm, 15 min) using the Ultra-Turrax® T 25 mixer (Ika, Labortechnik, Germany). The spontaneously formed multilamellar vesicles (MLV) were pressed through 200 nm size pore polycarbonate membrane to obtain unilamellar liposomes with the help of a Mini Extruder®

Lipofast extrusion device (Avestin Ottawa, Canada).

Characterization of liposomes in liposome dispersion and liposome gel. The diameter of vesicles, polydispersity index and zeta potential were determined by photon correlation spectroscopy using the Zetamaster S (Malvern Instruments, UK). The particle size was calculated from the autocorrelation function of the intensity of light scattered from particles, assuming spherical form of particles, medium viscosity of 0.89 mPa.s, and refractory index of 1.33. Liposome dispersions were diluted with PBS (pH 7.4) prior to the measurements, that is, 5 μL of the vesicle dispersions were diluted with 495 μL of PBS (pH 7.4). To obtain the zeta potential values of vesicles, 10 μl of vesicle dispersions were diluted with 990 μl of PBS pH 7.4.

To obtain the particle size and the polydispersity index (PDI) of liposomes incorporated in the hydrogel, 0.2 g of the hydrogel was diluted (1:10 w/w) with PBS (pH 7.4), mixed until a clear dispersion was obtained, and, afterward, the dispersion was centrifuged at 3000 rpm. Then, 50 μL of the supernatant were further diluted with 450 μL of PBS (pH 7.4) and analyzed. PDI was used as a value of a unimodal size distribution, which ranges from 0 (homogenous dispersion) to 1 (high heterogeneity). Each sample was measured three times and the mean value was determined.

Determination of pH value in the liposome dispersion. The pH value of the liposome dispersion was measured using a pH meter (HI 8417, Hanna Instruments, USA).

Preparation of Q_{10} gels

As a vehicle for the incorporation of the liposome dispersion Ultrez® 10 polymer was used since the compatibility of liposomes with carbomer gels was previously demonstrated [25, 26]. The gel was prepared by the following procedure: carbomer resin (0.8% w/w) was dispersed in distilled water in which propylene glycol (5% w/w), disodium edetate (0.1% w/w) and the preservatives (Phenonip® 0.2% w/w and Germal® II 0.3 % w/w) were previously added and left to wet for 30 min. The mixture was then neutralized by addition of 10% (w/w) triethanolamine under stirring (300 rpm, 5 min) using the Ultra-Turrax® T 25 mixer (Ika, Labortechnik, Germany), until a transparent gel appeared.

Incorporation of liposomes into the carbomer gel

The liposome dispersion was mixed into the gel by an electrical mixer (200 rpm, 5 min, Heidolph RZR 2020, Germany) and a liposome gel (G1) was obtained. The concentration of the liposomes in the gel was 10% (w/w, liposome dispersion/total), while the concentration of pure Q_{10} in the gel was 0.05% (w/w). The control gel (gel with free Q_{10}, G2) was prepared by first solubilizing Q_{10} (0.05% w/w) in water using Tween® 80 (0.25% w/w) as a solubilizer and then adding all other components, as described above.

pH determination of gels

The pH values were determined directly in gels at room temperature (HI 8417, Hanna Instruments, USA) and served to evaluate the chemical stability. Three measurements were performed for each sample and the mean value was calculated.

Rheological evaluation of gels containing liposomes

The rheometer (Rheolab MC 120, Paar Physica, Stuttgart, Germany) was used to determine flow properties of fresh (48 h old) gels with incorporated liposomes (G1) and without liposomes (G2). In order to predict their physical stability, the flow properties of these gels were also determined after subjecting them to five cycles of temperature stress (1 cycle: 24 h at 4°C, 24 h at 20±2°C and 24 h at 40°C). Measurements were performed at 20 ± 0.1°C by using the cone/plate MK 22 (radius of measuring cone 25 mm, angle of measuring cone 1°) measuring system. Continuous flow tests were carried out by increasing the shear rate from 0 to 200 s^{-1} and decreasing it back to 0 s^{-1}, each stage lasting 200 s. Under the same conditions the flow properties of the liposome gel and the control gel with free Q_{10} were examined. Three measurements were performed for each sample and the mean value was determined.

Determination of Q_{10} content in the samples by HPLC assay

The amount of non-degraded Q_{10} was determined quantitatively in the samples by HPLC analysis, 24 h after preparation. The HPLC apparatus consisted of HPLC pump Waters M600E (isocratically delivered mobile phase) and sample injector Rheodyne 7125i (injection volume: 10 μl). The investigation was performed at room temperature. The analytical column: Chromolith Performance RP-18e, 100 mm × 4.6 mm, 5 μm (Merck, Germany) and the detector: Spectral UV/VIS, PDA SPD - M10 A_{VP} (Shimadzu, Japan) were used. The flow rate was 2.1 ml/min and the UV detection was at λ = 275 nm. The used mobile phase was isopropanol : methanol (25 : 75). Three measurements were performed for each sample and the mean value was determined.

Visualization of liposomes in the liposome dispersion and liposome gel

Freeze fracture electron microscopy (FFEM) was used for the visualization of the liposomes in the liposome dispersion and liposome gel G1. Small amounts of the dispersion or gel were mounted on a gold specimen holder, which was placed between two copper preparation holders. The samples were then quickly frozen by plunge/freezing (Jet Freeze Device BAL/TEC, JFD 030, Liechtenstein) into liquid propane at -180ºC. The frozen specimens were kept in liquid nitrogen until mounting onto the sample holder. The latter was then placed into a freeze fracture device (Freeze Etching System, BAL-TEC, BAF 060, Liechtenstein). Samples were fractured and the fracture plane was replicated by evaporation of 2 nm platinum at an angle of 45º followed by 20 nm carbon at an angle of 90º. Replicas were removed from the freeze fracture device and cleaned in chloroform/methanol (1:1) mixture. Then, they were mounted onto copper grids and visualized using a transmission electron microscope (Zeiss EM 900, Zeiss, Germany).

Statistical analysis

Statistical analysis was carried out using One-Way Analysis of Variance. Significant differences were determined at $p < 0.05$.

RESULTS AND DISCUSSION

Characterization of the Q_{10}-loaded liposome dispersion

FFEM confirmed the presence of spherical liposomes in the liposome dispersion (Fig. 1). Liposomes containing Q_{10}, composed of unsaturated phospholipids (vesicles in fluid thermodynamic state) were characterized (Table 1) and the results of the particle size analysis showed that the mean size of the liposomes was 125.5 nm. Regarding the homogeneity, the low values of the polydispersity index 0.20 indicated homogeneous population of liposomes. Thus, the obtained liposomes were of small particle size and satisfactory homogeneity.

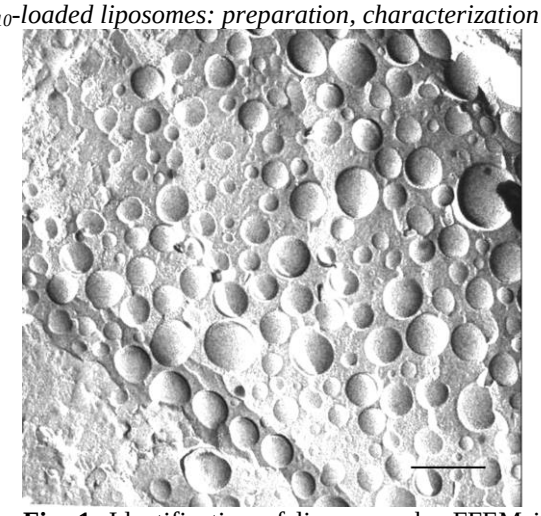

Fig. 1. Identification of liposomes by FFEM in the liposome dispersion after its preparation (scale bar = 100 nm).

As to the pH value, the results indicated that the liposome dispersion due to the use of buffer had a mild acidic pH value of 6.47, being desirable for liposomes for topical application and also because they show high chemical stability around this pH value. Namely, chemical hydrolysis of liposomal lipids at pH 6.5 occurs at the slowest rate. The pH value should always be above pH 4.5 as the pH value of 4.5 represents the critical lower limit when the degradation process of the vesicles may occur [27]. The stability of liposomes can also be predicted on the basis of the zeta potential value. As the liposome dispersion possessed a very high zeta potential of -63.9 mV (Table 1), the obtained Q_{10}-loaded liposome dispersion was classified according to the Riddick′s classification [28] into dispersions of high physical stability which do not show a potential to aggregate. Hence, the liposome dispersion containing Q_{10} could be considered as a physically and chemically stable dispersion. As to the Q_{10}-content, it was not significantly different from the declared Q_{10}-content of a 0.5% w/w dispersion (Table 1). In order to confirm the liposomes integrity in the liposome gel G1, the gel was analyzed by FFEM. As seen in the FFEM micrograph shown in Fig. 2, the liposomes were identified in the gel structure, indicating their compatibility with carbomer gel.

Table 1. Characteristic parameters of the liposome dispersion (LD), 24 h after preparation.

Sample	Mean size (nm)	PDI	Zeta potential (mV)	pH	Q_{10} content (%, w/w)
LD	125.5±0.2	0.20±0.001	-63.9±0.1	6.47±0.03	0.498±0.003

Characterization of the Q_{10}-loaded liposome gel and liposome-free gel

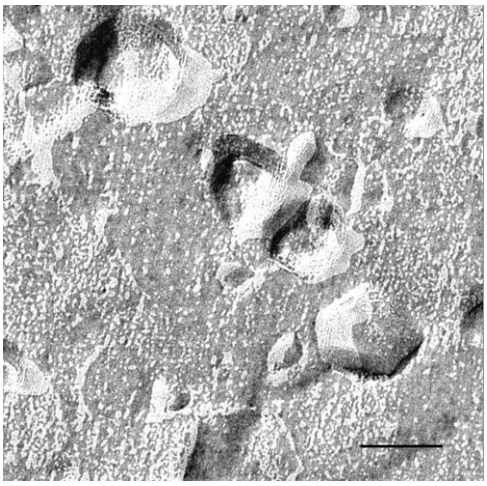

Fig. 2. Identification of liposomes by FFEM in the liposome gel G1 after its preparation (scale bar = 100 nm).

As to the characteristic parameters of the liposome gel (pH value, mean particle size and PDI of liposomes), they increased after the liposome incorporation into the gel (Table 2). As to the particle size and PDI value of liposomes in the gel, they increased compared to values in the liposome dispersion, but not remarkably, indicating compatibility of the liposomes with the carbomer gel. Regarding the pH value, its value of 6.12 for the liposome gel revealed that the gel was suitable for topical application. The same was observed for the liposome-free gel having a pH value of 6.0. The Q_{10}-content in the liposome gel and the liposome-free gel did not vary significantly from the declared Q_{10}-content of a 0.05 % w/w dispersion (Table 2).

Stability evaluation of the Q_{10}-loaded liposome gel and liposome-free gel

Rheological characterization of the gels. The rheological behavior of the gels was studied since it plays an important role in the mixing and flow characteristics of materials, their packaging into containers, physical stability and consumers' acceptability. The flow curves and the rheological parameters of the two examined gels obtained 48 h after their preparation are shown in Figs. 3 and 4 and in Table 3. Both samples showed a non-Newtonian behavior, since their viscosities were not constant, but changed as a function of the shear rate. Both gel formulations showed a shear-thinning behavior according to the Herschel-Bulkley model (excellent fitting $R > 0.999$) with continuously decreasing viscosity, thus indicating successive loss of polymer entanglement upon increasing shear stress [29]. Shear-thinning is a desirable property of semisolid dosage forms, since they should be "thin" during application and "thick" otherwise [30, 31]. The flow curves also showed a plastic behavior of the samples, since they displayed yield values, indicating that the gel network exhibited resistance to an external force before it started flowing [32]. The gel samples showed marginal thixotropy, since most carbomer polymer gels exhibit little or no thixotropy. As to rheological parameters (yield stress, minimal and maximal apparent viscosities), their values for gels examined 48 h after preparation are represented in Table 3. The yield stress can be used to evaluate the quality of a formulation and according to some authors it is the most reliable parameter for describing the stability [33, 34].

Table 2. Characteristic parameters of gel samples G1and G2 (mean size and PDI of liposomes, pH of gels), 48 h after their preparation and after the temperature stress test.

Sample labels	pH value of gels		Mean size of liposomes		PDI of liposomes		Q_{10}-content (%, w/w)	
	48 h	Stress test	48 h	Stress test	48 h	Stress test	48 h	Stress test
G1[a]	6.12±0.00	6.16±0.01	124.8±0.4	128.5±0.2	0.20±0.002	0.24±0.004	0.0496±0.002	0.0471±0.003
G2[b]	6.00±0.03	6.05±0.01	-	-	-	-	0.0499±0.002	0.0444±0.002

[a] G1 gel containing Q_{10}-liposome dispersion; [b]G2 gel containing free Q_{10}

Table 3. Yield stress values, maximal apparent and minimal apparent viscosities of gel samples G1 and G2 (mean, n = 3).

Sample labels	Yield stress value (Pa)		Maximal apparent viscosity (Pa) at 4.08 s^{-1}		Minimal apparent viscosity (Pa) at 200 s^{-1}	
	48 h	Stress test	48 h	Stress test	48 h	Stress test
G1[a]	61±0.8	75±5.8	50.8±1.3	50.3±1.7	2.93±0.08	2.96±0.08
G2[b]	51±5.8	74±4.5	33.6±0.8	36.3±0.3	2.26±0.01	2.38±0.02

[a] G1 gel containing Q_{10}-liposome dispersion; [b]G2 gel containing free Q_{10}

For topical preparations it is desirable to possess the yield stress not only in terms of good stability but also because it describes the flow behavior at small shear rates, i.e. before and after the application. The yield value of the fresh liposome gel G1 was 61 Pa, while for the liposome-free G2 gel it was 51 Pa (Table 3), indicating high stability of both gels, higher for gel G1. As to the minimal and maximal apparent viscosities, they describe different conditions of a structure, i.e. maximal apparent viscosity describes the system structure at rest, while the minimal apparent viscosity represents a measure of destruction of the gel structure. The values of these two parameters for gels G1 and G2 are represented in Table 3. Both gels possessed minimal and maximal apparent viscosities appropriate for semisolids aimed for topical application onto the skin.

Besides performing rheological measurements of the gels after their preparation, also analysis of the gels exposed to a temperature stress test was performed in order to determine the range of conditions under which the product will perform well, i.e. will remain physically stable. On the basis of the flow curves of the gels subjected to the temperature stress tests, which are shown in Figs. 3 and 4, it was concluded that the gels maintained plastic behavior after the temperature stress test, which is appropriate for topical use. In the case of gel G1, the flow curve did not change significantly, while the flow curve of the gel G2 changed when the gel was subjected to the temperature stress test. As to rheological parameters (Table 3), variations were observed in both gels exposed to the temperature stress test. It was evident that the yield stress values of both gels, significantly increased ($p<0.05$) during the temperature stress test. This can be explained by an increasing number of bonds in the polymer network which yields a higher physical stability. Thus, their stability did not decrease due to the temperature changes and based on the data on yield stress obtained by this test a high stability of gels was assumed. Further, the minimal and maximal apparent viscosities of the gel G1 did not change significantly ($p>0.05$) after the temperature stress test compared to their initial values (Table 3). In contrast, in the case of gel G2, the temperature stress test led to a significant increase ($p<0.05$) of the minimal and maximal apparent viscosities. However, the value of minimal apparent viscosity was higher only for 5 %, and the value of maximal apparent viscosity was higher for 8%, compared to their initial values. This increase in viscosity values of gel G2 could be explained by changing, e.g. by strengthening the gel structure due to forming new bonds. However, liposome gel G1 exhibited less extensive changes in rheological parameters and hence in the structure compared to gel G1, when subjected to the stress test. Further, liposome gel G1 possesses higher yield stress after preparation, indicating higher physical stability than the gel G2.

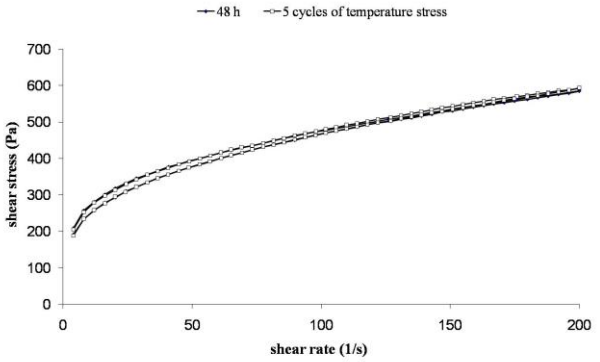

Fig. 3. Flow curves of the gel G1, 48 h after preparation and after the temperature stress test.

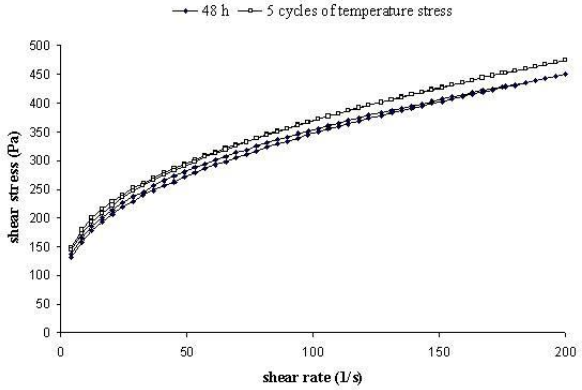

Fig. 4. Flow curves of the gel G2, 48 h after preparation and after the temperature stress test.

On the basis of these results it was concluded that both gel samples remained physically stable after the temperature stress test and that they did not exhibit relevant changes in their rheological properties which could make them unsuitable for topical use onto the skin. As to the liposome gel, the obtained results from rheology measurements revealed that the addition of liposomes into the gel neither decreased the stability of the carbomer gel, nor the stability of liposomes, as shown by PCS (see next section), indicating compatibility of liposomes and carbomer gel.

pH value of gels and mean particle size and PDI of liposomes in liposome gel

The temperature stress test did not lead to a significant ($p>0.05$) change in the pH values of the liposome gel and liposome-free gel (Table 2) and the obtained pH values were appropriate for the application onto the skin. Insignificant pH changes in the samples indicated that total neutralization of

carboxylic groups of polyacrylic acid occurred by the addition of TEA during the preparation of gels, as well as that no chemical changes took place in the samples during the temperature stress test. As to the particle size and PDI of liposomes in the gel after subjecting it to the temperature stress, no significant change ($p>0.05$) was measured. Hence, no aggregation of liposomes in the liposome gel occurred.

Stability of Q_{10} in Q_{10}-loaded liposome gel and liposome-free gel

The Q_{10}–content values of gels obtained after the temperature stress test are shown in Table 2. The statistical analysis revealed that the Q_{10}-content significantly decreased ($p<0.05$) in the gels. The Q_{10}–content in the liposome gel after the temperature stress test was 95% of the initial content, this being a satisfactory result. As to the liposome-free gel, the Q_{10}–content was found to be 89% of the initial content. The higher stability of Q_{10} in the G1 gel in comparison to the G2 could be explained by the greater ability of the liposome gel to protect Q_{10} from degradation, as Q_{10} is incorporated in the liposomes, i.e. in phospholipid bilayers, compared to plain gel without liposomes. Q_{10} showed a significantly higher ($p<0.05$) stability in the liposome gel G1 than in the liposome-free gel G2.

CONCLUSION

This study demonstrated the feasibility of preparing a Q_{10}-loaded liposome dispersion from non-hydrogenated soybean lecithin and a Q_{10}-loaded liposome gel. The incorporation of the Q_{10}-loaded liposome dispersion into the liposome gel did not lead to instability or degradation of liposomes. The obtained liposome gel, being a semisolid formulation, was in contrast to the liquid liposome dispersion, suitable for topical application onto the skin and its rheological properties were appropriate for this kind of application. In order to predict its long-term stability, the liposome gel was subjected to a temperature stress test. The liposome gel maintained a non-Newtonian plastic flow behavior without significant thixotropy after the temperature stress test, and the rheological parameters did not significantly change, indicating its physical stability. In addition, neither the liposome size nor PDI of liposomes changed dramatically in the liposome gel upon subjecting it to extreme storage conditions, confirming the vesicle stability (i.e., no agglomeration or aggregation of liposomes occurred). Furthermore, the Q_{10}-content in the liposome gel changed, but to an acceptable level, keeping in mind the extreme test conditions being used. This result clarified that extreme storage conditions should be avoided. Coenzyme Q_{10} was more stable in the liposome gel than in the liposome-free gel. Thus, the Q_{10}-loaded liposome gel could be considered, according to the results obtained after the temperature stress test, as a physically and chemically stable gel appropriate for topical use.

This liposome gel is aimed to be used as it is or as an enriched formulation with additional cosmetic actives (emollients, moisturizing agents, etc.), which should be investigated in future studies, as a cosmetic anti-ageing preparation. Not only Q_{10}, but also liposomal constituents (phospholipids) are beneficial for skin care. This study showed the feasibility of preparing a Q_{10}-loaded liposome gel serving as a delivery system of Q_{10} to the skin. The next step will be the evaluation of the penetration enhancing ability of this liposome gel, as well as an *in vivo* investigation of its anti-ageing effect.

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